Influenza virus infection causes both recurrent annual epidemics and more serious pandemics that spread rapidly. When influenza A viruses are transmitted to epithelial cells, the viruses replicate in the epithelial cells, and macrophages and leukocytes respond to produce chemokines and cytokines. In addition, myeloperoxidase (MPO) in neutrophils is associated with cellular damage upon influenza viral infection. Influenza virus strain H5N1 infection accompanied by acute respiratory distress syndrome (ARDS) starts with high fever, then proceeds to serious respiratory failure. In our study, first, we focused on the response of cytokines, chemokines, and MPO activity in ARDS pediatric patients infected with H5N1. Second, we examined cytokine/chemokine production in A549 human epithelial cells infected with influenza A/H1N1 virus (PR-8) or nonstructural protein 1 (NS1) plasmid in vitro.

In the plasma, levels of IL-12p40 and TNFR2 in the H5N1-positive group were significantly higher than in the H5N1-negative group. In the nasopharyngeal aspirate (NPA), the concentration of sIL-6R in the H5N1-positive group was significantly higher than in the H5N1-negative group. In addition, MPO activity in plasma was significantly higher in the H5N1-positive group. These results suggest that sIL-6R may be a major contributor in the nasal space associated with lung injury induced with H5N1 infection. Moreover, IL-12p40 and TNFR2 may be produced from the pulmonary space or endothelial cells to circulate in the blood, and MPO may be released into the blood from activated neutrophils, resulting in the lung injury.
Second, *in vitro* study suggests that TNF-α and RANTES were predominantly produced from the A549 epithelial cells infected with PR-8 virus. siTNF-α down-regulated RANTES expression and secretion of RANTES, IL-8, and MCP-1. In addition, siRANTES suppressed interferon (IFN)-γ expression and the secretion of RANTES, IL-8, and MCP-1. Furthermore, administration of TNF-α and RANTES promoted elevated secretion of RANTES, IL-8, and MCP-1 production in uninfected cells, strongly suggesting that RANTES produced by TNF-α may regulate following increase of IL-6, IL-8, MCP-1, and IFNs levels in the initial step. In the next step, the cells transfected with viral NS1 plasmid showed production of a large amount of IL-8 and MCP-1 in the presence of the H$_2$O$_2$-MPO system, suggesting that NS1 of PR-8 may induce a “cytokine storm” from the epithelial cells when an H$_2$O$_2$-MPO system exists. These findings are similar to the result that MPO promotes the development of lung neutrophilia and indirectly influences subsequent chemokine and cytokine production in the lung.

Influenza virus infection induces a cytokine storm in lung epithelial cells, which is associated with NS1 of influenza virus when the H$_2$O$_2$-MPO system acts.